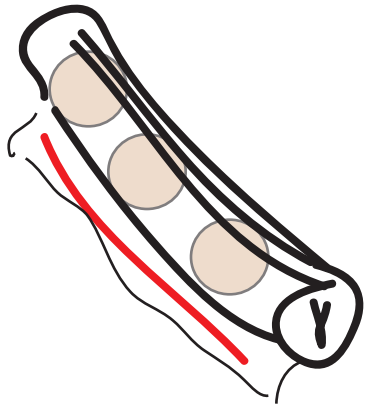


## E4.5-5.0 embryo dissection

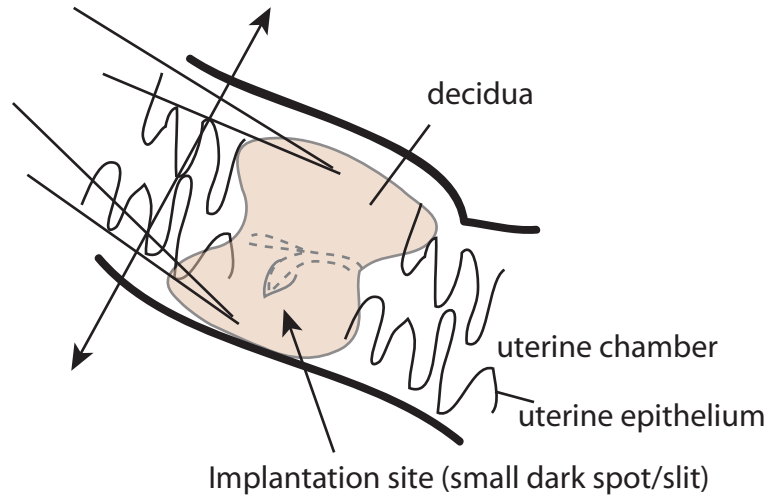
Yojiro Yamanaka  
Rossant lab, Hospital for Sick Children

60mm dish  
Two pairs of No.5 or No.55 forceps  
32 G needles  
1ml syringes (holders for needles (remove plungers))

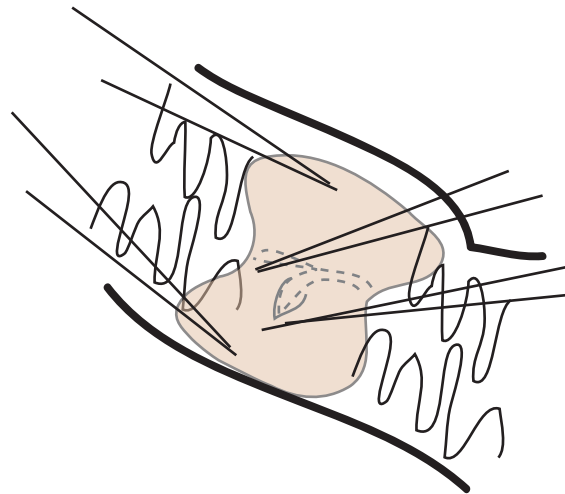
Dissection scope equipped with a bottom light  
PBS with Ca, Mg  
or F12 with 15mM Hepes (7.0) and 5-10% FCS



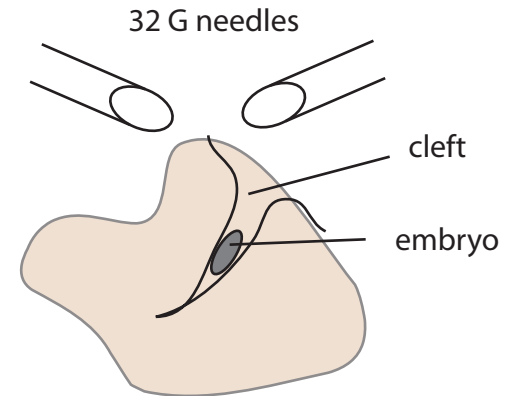
Take out one horn of uterus.



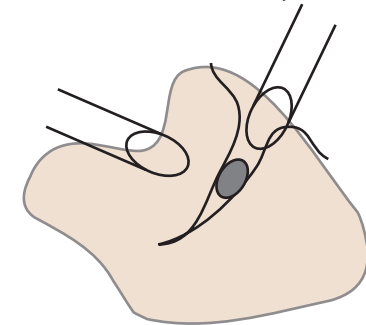
Stretch and flatten out the uterus using forceps and locate decidua. Using a bottom light, locate a small dark spot/slit in the decidua.



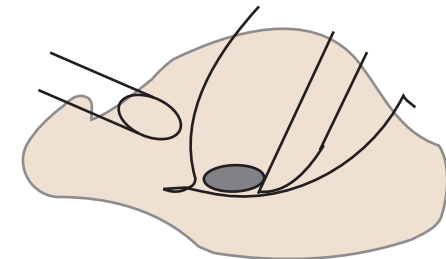
Pinch off the implantation site with surrounding decidua tissue.



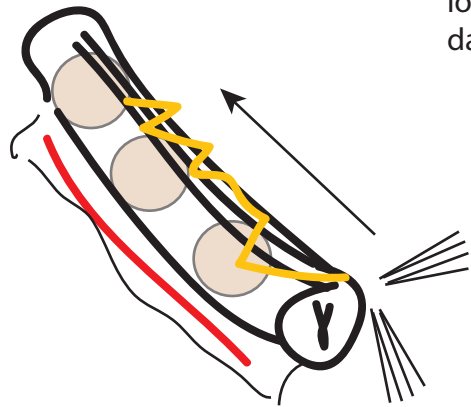
Locate a cleft where an embryo is implanting. You cannot see the embryo this point.



Insert a tip of a needle and make a slit in the decidua along the cleft.



Expose the implantation site (now you should see the embryo!!). Gently scratch off the embryo from the decidua using the back side of the needle.



Cut the anti-mesometrium side of muscles along the uterus with forceps to expose the inside.

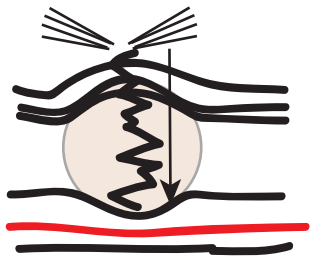
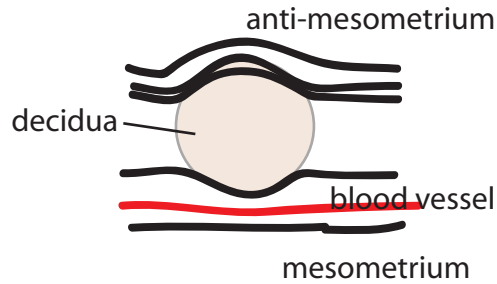
# E5.5 embryo dissection

Yojiro Yamanaka  
Rossant lab, Hospital for Sick Children

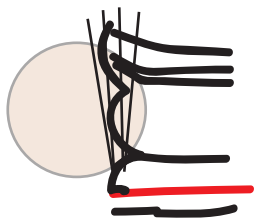
60mm dish  
Two pairs of No.5 or No.55 forceps  
32 G needles  
1ml syringes (holders for needles (remove plungers))

Dissection scope equipped with a bottom light  
PBS with Ca,Mg  
or F12 with 15mM Hepes (7.0) and 5-10% FCS

## Taking out a decidua from uterus

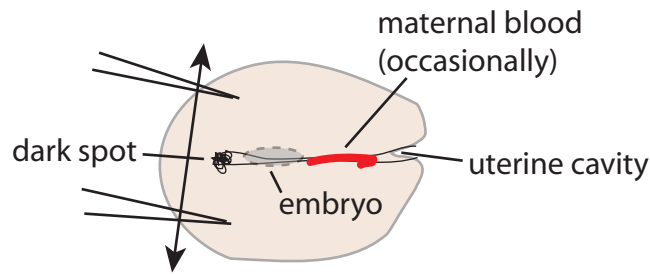


Pinch and cut uterus muscles using forceps from the anti-mesometrium side.

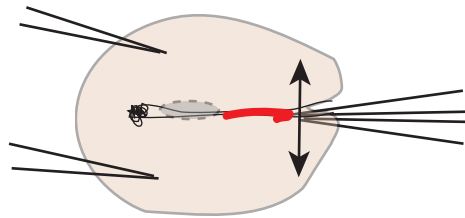


Pinch off the decidua with forceps

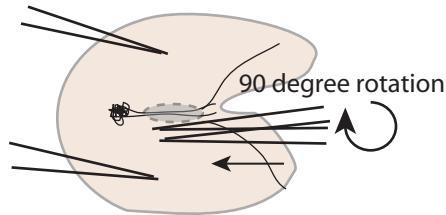
## Taking out an embryo from a decidua



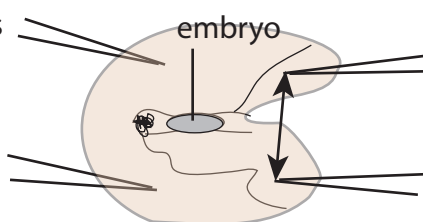
Hold and flatten out the decidua gently with left forceps. Using a bottom light, locate a utrine cavity,maternal blood (no blood at early E5.5) and a dark spot at the opposite side of uterine cavity. The embryo sits next to the spot.



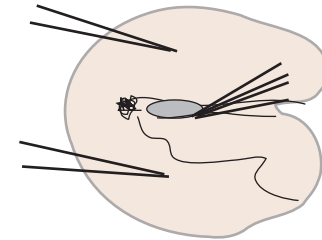
Open up the uterine cavity and flatten out the decidua using right forceps.



Rotate right forceps 90 degree (forceps move vertically) and insert the bottom tip of forceps at the off center of the dedcidua adjacent to the embryo. Snip off the decidua and expose the implantation site.

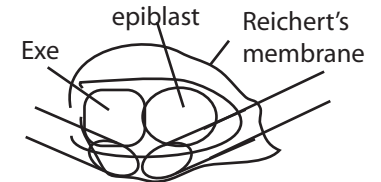


The embryo is almost invisible. We recognize it as its texture and reflection to the light. You may see it as a small bulge along the uterine cavity. You need to calibrate your eyes to see it!

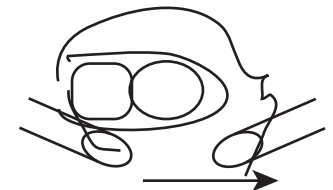


Using the back side of forceps, the embryo is gently removed from the decidua.

## Removing the Reichert's membrane



Hold 32G needles as beveled sides up. Put both tips of needles closely in the space in yolk sac.



Gently pull one of needles.